

TED ANKARA COLLEGE FOUNDATION

HIGH SCHOOL

Investigating the effect of different amounts of irrigation level on the protein amount of grains of Cicer arietinum indicated by the Bradford method

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Abstract

Cicer Arietinum as commonly called as chickpeas are known that they are highly content protein. A half cup serving of *Cicer arietinum* will typically provide 7g of protein. The high protein and low fat content of chickpeas makes them attractive to vegetarians, dieters and others interested in a healthy lifestyle. In this experiment the aim of the experiment is investigating the effect of different amounts of irrigation level on the protein amount of grains of *Cicer Arietinum* indicated by the Bradford method. Therefore, my research question is “what is the effect of different amounts of irrigation on the protein amount of grains of *Cicer arietinum* indicated by the Bradford method?”

Firstly, 5 groups with 5 pots in each group of *Cicer Arietinum* was planted and raised to eliminate the genetic factors with different levels of irrigation. After the grains of the *Cicer Arietinum* has grown, I took 5 grains in each pot and isolated the proteins and evaluate the protein amount with the help of a computer programme called Bradford method. From the data obtains I came with the result that the negative effects of irrigation to agriculture since *Cicer Arietinum* is a summer plant it needs less water than winter plants. The data that obtained supported the hypothesis that different levels of irrigation on *Cicer arietinum* creates a significant change in the amount of protein that their grains contain.

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1. Introduction

The first time I thought about considering this subject, my grandmother was making a dinner with vegetables and she added some chickpeas to the meal. After that I realized that she puts chickpeas almost every meal she made. I wondered whether she includes chickpeas because its healthy for human beings or just for its taste, so when I asked her why she said to me chickpeas are really healthy for us because they contain great amounts of protein. I thought it was an interesting subject for me on my thesis to investigate in whether every chickpea have same amount of proteins in every environmental condition. So I decided to work on this subject as investigating the effects of irrigating on the amount of protein in *Cicer arietinum*.

Proteins are large biological molecules consisting of one or more chains of amino acids. They perform a vast array of functions within living organisms, including catalyzing metabolic reactions, replicating DNA, responding to stimuli, and transporting molecules from one location to another. Proteins differ from one another primarily in their sequence of amino acids, which is dictated by the nucleotide sequence of their genes, and which usually results in folding of the protein into a specific three-dimensional structure that determines its activity. ^[1] There are two groups of amino acids according to their reception. There is a group of amino acids that an organism can synthesize in its cell and the other group is an amino acids called “essential amino acids” which can not be synthesized natively by the organism and therefore must be taken in its diet. Therefore a diet that contains essential amino acids is a very important nutrient for the organism to provide essential amino acids and not suffer from their deficiency. And *Cicer arietinum* is a rich nutrient as an included amount and type of protein so it is an important aliment for human beings for consuming them.

Cicer arietinum grows to between 20–50 cm (8–20 inches) high and has small feathery leaves on either side of the stem. They are a type of pulse, with one seedpod containing two or three peas. It has white flowers with blue, violet or pink veins. According to the Centers for Disease Control, a ½ cup serving of *Cicer arietinum* will typically provide 7g of protein. The high protein and low fat content of chickpeas makes them attractive to vegetarians, dieters and others interested in a healthy lifestyle. When combined with other healthy foods, *Cicer arietinum* can be used to provide our body with the complete profile of amino acids needed to make the protein that it needs for proper health ^[2] *Cicer arietinum* includes rich source of zinc, folate and protein and are low in fat and most of this is polyunsaturated. ^[3] Moreover, it provides many amino acids but is typically low in methionine and histidine. ^[4]

There are different factors that could affect amount of protein *Cicer arietinum* includes. According to the researches which investigates the effects of rainfall and irrigation on the grain protein, with the irrigation increases, the rate of the grain point and the grain efficiency increases to a certain point and

as a result the unit area of the protein efficiency also increases. ^[5] Therefore amount of water as an external factor changes amount of protein in *Cicer arietinum*. It is not that surprising since water is one of the essential substances for a plant as for a living creature. The need of water depends on different factors such as climate zone, drought or excessive rain. All plant need different amount of water to grow. Winter plants want high rate of irrigation since they found water more easily than a drought area plant and summer plants like *Cicer arietinum* want less amount of water than winter plants as they found water harder and it is called an adaptation. As a result it could be said that amount of irrigation has a precise importance on the level of protein in the grains of *Cicer arietinum*. To conclude, with the increasing level of irrigation, might increase protein efficiency as well as too much increase in irrigation would be harmful since it is a summer plant.

In the light of these information, aim of the experiment was determined as to investigate the effects of different levels of irrigation, which is important in agriculture, on protein amount of *Cicer arietinum* grains which would be consumed by human beings as a part of healthy diet. In that respect my research question is “what is the effect of different amounts of irrigation on the protein amount of grains of *Cicer arietinum* indicated by the Bradford method?” In order to perform a controlled and manageable investigation, the experiment will be done in room temperature and in same medium and the same type of *Cicer arietinum*. In the earth there are two subspecies of chickpeas which are cultivated according to its grain size, shape and color. Those are the “Desi” type and the “Kabuli” type chickpeas. For the experiment the Kabuli type chickpea plant is used rather than Desi type due to its advantages. Also irrigation was done at the same time, once a day with different volumes of distilled water.

2. Hypothesis

Cicer arietinum need a subtropical or tropical climate zone with more than 400 millimeters of annual rainfall. They can grow in temperate climate but yields will be much lower. Need for less water of *Cicer Arietinum* is not as much as a winter plant since it is a summer plant. On the other hand when the amount of rainfall increases unit area of protein efficiency increases as well. By dehydration synthesis amino acids are linked to each other to form proteins so there can be said that water plays an important role in protein synthesis. As a result it could be hypothesized that “different levels of irrigation on *Cicer arietinum* creates a significant change in the amount of protein that their grains contain”. According to the hypothesis that concluded there can be a null hypothesis which is “different irrigation amount does not have any effect on the protein production of the plant *Cicer arietinum*”.

3. Method Development

3.1) Preparation

The aim of the experiment was determined as investigating the effects of different levels of irrigation on protein amount of grains of *Cicer arietinum*. Therefore my research question is “what is the effect of different amounts of irrigation on the protein amount of grains of *Cicer arietinum* indicated by the Bradford method?” This question leads to the hypothesis “different levels of irrigation on *Cicer arietinum* creates a significant change in the amount of protein that their grains contain.” Before starting the experiment with the specie of the plant *Cicer arietinum*, the volume of water used in irrigation determined as an independent variable. As I investigate the amount of protein, the dependent variables of my experiment would be the amount of proteins in grains of *Cicer arietinum*. In order to reach my aim I kept all the other factors constant.

Every plant has its own amount of protein and rate of protein synthesis. Because the rate of protein synthesis change from plant to plant I will use the same type of seeds to avoid the different rate of reaction. I choose the sub specie Kabuli rather than Desi as it mentioned in the introduction for its advantages. As a *Cicer arietinum* or a chickpea Kabulies’ grains have larger size and spearhead shape with white or light-cream color rather than green Desies’. Moreover, Kabulies are more adopted to temperate regions (like Turkey) and not resistant to the cold weather which is appropriate for our country.

To make the experiment properly I decided to plant seeds of *Cicer arietinum* Kabulies’ to control genetic differences. There were five groups each consist of five pots and each pot have the same type of 5 seeds. I preferred 5 seeds in each pot rather than 1, in case, there were some problems in germination because in some cases a seed cannot germinate. Each group irrigated once a day with different volumes of distilled water.

Until the first grains are taken shape there are two stages occurred which are germination stage and growing stage. For germination stage;

Type and mass of soil that are placed on pots was an important controlled variable for my experiment. Since plants take important mineral and vitamins from soil it is important to make them take same amount of minerals and vitamins. To keep the concentration of minerals, in addition to aeration and water holding capacity constant I needed to keep the mass and type of soil constant. I used the clay soil type because of the tiny size of its particles and its tendency to settle together, air can pass through its spaces. Because it’s also slower to drain, it has a firmer hold on plant nutrients. Clay soil is thus

rich in plant food for better growth. By the help of weighing machine 100 gr soil was used for each pot which was appropriate with the identical pot sizes (basket type with 35 cm diameter and 30 cm depth)

The pH of the soil also kept constant. The best pH values for nitrogen, phosphorus and potassium to uptake by plants are 6,5-7,5. If phosphorus is lower than the 6,0 pH value and aluminium and iron is higher than the 7,5 pH value they make bonds with carbon that is why it is hard to uptake by plants.⁽⁶⁾ Clay type of soil also appropriate with these values and controlled with pH test paper.

Timing for irrigation and the type of water kept constant too. Because in the nature plants take underground water or rain to obtain water source, the plants are given distilled water in the experiment to make the factors that plays role for their growth to resemble their natural environmental factors. Also plants are irrigated in the morning because the mornings are chiller than the day time and there will less water loss by evaporation. Volumes that were chosen for irrigation was selected according to the size of the pots.

The other factor that kept controlled was temperature. There are certain temperatures that a seed could grow best. The temperature of the soil was kept at 15°C because it is the most available temperature for a seed to be kept both warm and moist. And the water in the soil is not evaporate as much as at the room temperature.

For the growing stage in addition to the controlled variables of germination stage other factors were also considered.

First, light intensity kept constant. Light intensity plays a huge role in plant development. Plants take solar energy to make their own food (photosynthesize), ATP and protein synthesis. By placing the pots in the same place (near window, in the same line 30 cm apart from each other, on the greenhouse) it provides plants to take same light intensity and wavelength.

For growth to occur, photosynthesis must be greater than respiration. Slow growth can be resulted in low temperatures therefore, at low temperatures photosynthesis is slowed down. Since photosynthesis is slowed, growth is slowed, and this results in lower yields. ⁽⁷⁾ Therefore, I placed the plants in the same room and use a thermometer to observe the temperature of the room (as 23°C) so I can interfere and kept it constant .

As another point for conducting and plausible experiment I used a laboratory of an university for my experiment and make 5 trials in order to get more accurate and precise outcome. 5 grains were taken from each pot from same sprit because the computer will read the results more accurately since there is more protein in 5 seed. For isolating the proteins, grains of *Cicer arietinum* were divided into 1-2 mm pieces to increase their surface area which enhanced the effect of liquid nitrogen for pulverizing the pieces. For evaluating the protein amounts I will be use the method of Bradford (aka Victor3). Lysis

buffer with same concentration and amount as mentioned in the materials and procedure section were used as destroying the cell membrane of the cells to reveal the cells protein. Centrifuge, sonification and ultrasonification homogenizator with 40 amplitude were used to isolate lefted proteins from the cells and expose the proteins. Lysis buffer, sonification, ultrasonification and centrifuge were the other controlled variables during the isolation of the proteins. For evaluating the protein amounts I used the method Bradford (aka Victor 3) based on a computer programme. There were other methods such as Lowry procedure and Elisa. Lowry based on a biochemical assay which is for determining the total amount of protein in any kind of solution. The protein concentration is displayed by the change of colour of the sample solution in comparison with protein amount that can later be measured y colorimetric techniques. The other method, Elisa, is based on an analytic biochemistry assay that uses a solid phase enzyme immunoassay. Since both of the procedures were above my knowledge and my ability to use technology I preferred to do the experiment with Bradford method.

3.2) Materials

- Lysis buffer
 - NaCl 0,15M 0,4383g (AmrescoX190)
 - EDTA 5mM 0,093g (Biorad 161-0728)
 - Tris-HCL 50mM 0,394g (Sigma T3253)
 - Np40 %1 500uL (Applichem. 1694,0250)
- Ultra sonification homogenizer
- 15 identical pots
- 1 pack of raw chickpea
- 5 chickpea grain in each group (5x5=25 grains)
- A muller (for physical distruption of grains)
- Centrifugal machine
- X25 tube with 2 mL
- Sensitive weighing machine
- 300 mL liquid nitrogen
- 200ug/ml, 400ug/ml, 600ug/ml, 800ug/ml, 1000ug/ml standarts (x5 for each one)
- 5X Biorad Protein Assay Dye Reagent consantrated paint
- Microplate with wells
- A vortex machine
- 1 plate with 20 cm depth with full of ice

3.3) Procedure

1. Buy 1 pack of 'Kabuli' *Cicer arietinum* that is raw and not seen any procedure
2. Take 5 identical pot and plant 5 *Cicer arietinum* seed in each pot.
3. Label the pots 10mL, 60 mL, 110 mL, 160 mL and 210 mL.
4. Irrigate the pots everyday with the volume of distilled water that has been written in the label at the 7 am.
5. Continue the step 4 until the chickpeas are fully grown and give their first product
6. Take 5 grains of *Cicer arietinum* from one of the pots labeled with 10 mL of water.
7. With a help of knife divide the grains into small pieces (1-2mm)
8. Measure the tare of 2mL tubes.
9. Pulverize the small pieces with the help of liquid nitrogen and beat them in a muller until they fully pulverized and put them in the 2mL tube without waiting.
10. Measure the 2mL tube again and minus the tare from the total weight and find the weight of the pulverized grain.
11. Put lysis buffer in the tube 2.5 times (mg- μ L, g-mL) than the weight of pulverized grain that is found in step 5 and vortex (1,5 second) it.
12. In sonification homogenizator with intensity of 40 amplitude sonificate the tube for 1-2 seconds (long period of sonification would harm the proteins) and .
13. Centrifuge the homogenized grains for 45 minutes in 14000xg.
14. Take the supernatant carefully from the centrifuge machine and pellet discarded

15. Measure the protein amount with the help of a laboruant in a computer programme which is Bradford method and divide the result with 5.
16. Repeat the steps 6-17 for 4 more times with the other 10 mL pots.
17. Repeat steps 6-16 for the pots labelled with 60, 110, 160, 210 mL

4. Analysis

4.1) Raw Data Table

Groups	Trials	Irrigation Amount (±0,1 mL)	Growth temperature (±0,5°C)	Seed type	pH of the soil (± 0.1)	Protein amount that each C.arietinum (±0.001)	Type of water	Duration of centrifuge (± 5 min)	Timing of irrigation (± 5 min)	Germination temp (±0,5 °C)	Distance of pots from each other (±1cm)	Intensity of sonification (Amp) (±0.5amp)	Amount of soil (±0.5 gr)	Duration of vortex (sec) (±0,05sec)	
Group 1 (10 mL)	Trial 1	10	25°C	Kabuli	7,1	95,24678215	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial2	10	25°C	Kabuli	7,1	95,24932754	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial3	10	25°C	Kabuli	7,1	95,25984356	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial4	10	25°C	Kabuli	7,1	95,24246397	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial 5	10	25°C	Kabuli	7,1	95,24415362	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
Group 2 (60 mL)	Trial 1	60	25°C	Kabuli	7,1	72,2378354	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial2	60	25°C	Kabuli	7,1	72,2309834	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial3	60	25°C	Kabuli	7,1	72,2378453	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial4	60	25°C	Kabuli	7,1	72,2312498	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial 5	60	25°C	Kabuli	7,1	72,2336745	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
Group 3 (110 mL)	Trial 1	110	25°C	Kabuli	7,1	70,72253691	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial2	110	25°C	Kabuli	7,1	70,73561267	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial3	110	25°C	Kabuli	7,1	70,72784635	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial4	110	25°C	Kabuli	7,1	70,72356409	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial 5	110	25°C	Kabuli	7,1	70,72356472	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	

Group 4 (160 mL)	Trial 1	160	25°C	Kabuli	7,1	69,59367582	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial2	160	25°C	Kabuli	7,1	69,59846576	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial3	160	25°C	Kabuli	7,1	69,58726452	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial4	160	25°C	Kabuli	7,1	69,59645098	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial 5	160	25°C	Kabuli	7,1	69,59123095	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
Group 5 (210 mL)	Trial 1	210	25°C	Kabuli	7,1	63,9074659	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial2	210	25°C	Kabuli	7,1	63,9176492	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial3	210	25°C	Kabuli	7,1	63,9187452	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial4	210	25°C	Kabuli	7,1	63,9074659	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	
	Trial 5	210	25°C	Kabuli	7,1	63,9065248	Distilled	45 min	At 7 am	15°C	30 cm	40 amp	400 gr	1,5 sec	

Table1: The raw data table of dependent variables, independent variables and the controlled variables of amount of protein in grains of *C. arietinum* irrigated with different volumes of water.

4.2 Descriptive statics

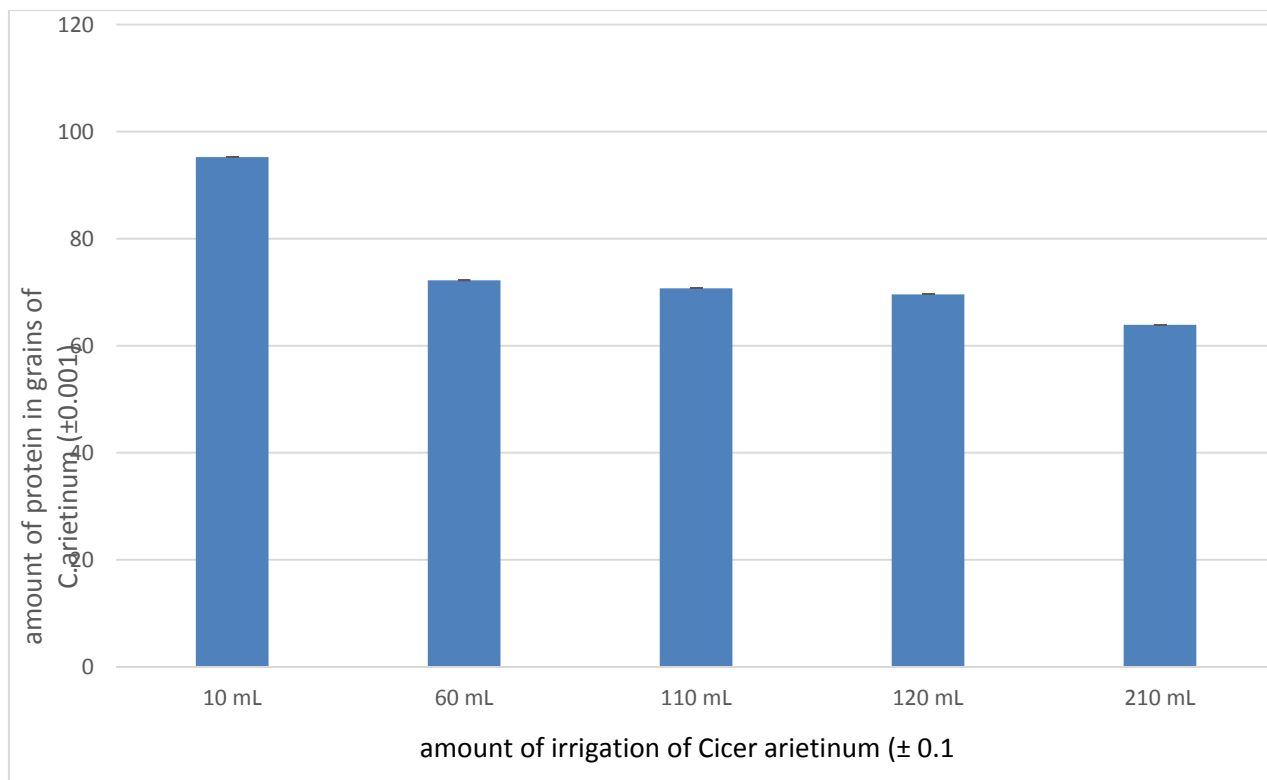
	10 mL	60 mL	110 mL	120 mL	210 mL
Mean	95,24851417	72,23431768	70,72662495	69,59341761	63,9115702
Standard Error	0,003062971	0,001512638	0,002426076	0,001967153	0,002716445
Median	95,24678215	72,2336745	70,72356472	69,59367582	63,9074659
Mode	--	--	--	--	63,9074659
Standard Deviation	0,006849012	0,00338236	0,00542487	0,004398688	0,006074156
Confidence Level(95,0%)	0,008504172	0,004199755	0,006735866	0,005461692	0,007542061

Table2: The table of mean, median, mode and standart deviation of the trials done to demonstrate the protein amount of the group 10,60,110,160 and 210 mL

4.3) The Anova Table

In the experiment, there were more than one group in independent variables so I used Anova (Analysis of Variance). In basic the analysis of variance ratios the variability between the groups to variety between individuals within groups. The purpose of the anova is to identify the differentiation between the groups are whether bigger or not from the differentiation between the individuals.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2929,692464	4	732,423116	25427407,91	9,5076E-67	2,866081402
Within Groups	0,000576089	20	2,88045E-05			
Total	2929,69304	24				



Graph1: Graph of protein amount versus volume of water.

5. Evaluation and Conclusion

The aim of this experiment was investigating the effects of the level of irrigation amount on the protein amount that a plant *Cicer arietinum* grains that they produce.

The results that I have found from the experiment based on the research question “What is the effect of different amount of irrigation on the protein amount of grains of *Cicer arietinum* indicated by the Bradford method?” supported the hypothesis that different levels of irrigation on *Cicer arietinum* creates a significant change in the amount of protein that their grains contain. When we look at the results, we see that there are not huge amount of differences between groups but still there is more amount of protein in the group with less irrigation amount and there is less amount of protein in the group with more irrigation amount. For determining whether there is a significant mean difference between groups ANOVA was used since there were more than two groups. The purpose of the ANOVA is to identify the differentiation between the groups are whether bigger or not from the differentiation between the individuals. As a result P-value was smaller than 0.05. Therefore there is a statistically important mean difference between groups

$$0,5 < \text{sig}=0,000$$

It can be said that the differentiation between groups are bigger than the differentiation between individuals. As a result “different levels of irrigation on *Cicer arietinum* creates a significant change in the amount of protein that their grains contain” can be accepted and “different irrigation amount does not have any effect on the protein production of the plant *Cicer arietinum*” can be rejected. The more irrigated plant had the less amount of protein and the less irrigated plant had the more amount of protein.

The average protein amount of the 5 seeds of each group was found 3809.975686, 2889.224456, 2829.0986796, 2783.9058008 and 2556.374404 10, 60, 110, 160, 210 mL respectively. Also standart error for each group was low so it could be said that the statistic were approached the actual value and the datas are representative of the actual values. There is a great amount of decline in the protein amount between the group irrigated with 10 mL and 60 mL. After the group 60 mL the amount of difference in the protein amounts between groups were not decreasing that much. So it can be said that since *Cicer arietinum* is a summer plant it is more adapted to the drought areas and could have more protein with less water. So results revealed negative effects of irrigation in agriculture it is important to consider this fact and prevent over irrigation to increase protein rate of *Cicer arietinum*. The optimum rate is so critical since there is not much change after 60 mL of water.

While doing the experiment there were some errors and limitations that could affect the data gathered from the experiment.

Since the sonification and ultrasonification produce sonic waves in really high frequencies and in a frequent way, I thought the waves that produced might be harmful for the monomers of the protein. When I investigate my concern I asked the scientists and protein analyzers in the laboratory, I found out that my concerns are true. But they said that the all methods while synthesizing the protein could harm the monomers and it would affect the counting a little. For getting the results more precisely the isolation of proteins from the grains could be done slower and sonification and ultrasonification could not be used for not to harm monomers. Since the sonification and ultrasonification will not be use the centrifuge could be used for longer period of time and this method will not harm the monomers.

Because of *Cicer arietinum* is a summer plant, the experiment should have been carried out during summer. By this way, since the plants will get more sunlight, and no bad weather conditions such as snow or strong wind, they would grow more easily so it would be more easy to observe the plants while they were growing. Also, since winter is not the season of the *Cicer arietinum*, choosing winter plant would be easier to observe and doing the experiment.

Another point is the genetic factors and the enzymes that a grain of the plant might be different from each other. Because of the genetic factors, while a grain could have great amount of enzymes, the other grain could not have as much as the other. Therefore the amount of protein might be variable apart from the irrigation amount. To minimize the genetic factors I used the grains of one *Cicer arietinum* and I planted the grains than I used the grains of the new chickpeas. Therefore I minimized the effects of the genetic factors.

Even though there were some errors, I could find an answer of my question. The plants in groups which have irrigated less have more proteins and the plants in groups which have irrigated more have less protein. This is also accurate with literature as it is a summer plant. So it could be said that irrigation has a precise importance on amount of protein in grains. People who consume *Cicer arietinum* in their diets should be aware of this as well as farmers. Especially vegetarians should care to consume *Cicer arietinum* from a place that have less annual rainfall.

As a suggestion for further investigation the effect of heat during cooking can be searched. Since I investigate the amount of protein that *Cicer arietinum* has, I searched and worked about protein. Then I thought people never eat chickpeas raw, they always boil them, cook them but sometimes heat can damage proteins. Therefore a new question arise “What is the optimum temperature that a chickpea can take without disrupting its proteins?” To answer the question the same method could be done but after heating the chickpeas in different temperatures.

Besides from that question I also would want to investigate other beneficial effects of *Cicer arietinum* apart from other vegetables. If there are any beneficial effects apart from other plants, *Cicer arietinum* would be an important source of nutrient to gain that effects. They would be isolated from the *Cicer arietinum* with the similar method to isolate the protein that has been used in the experiment. There are some medicines that have been made from plants so if beneficial effects of the *Cicer arietinum* would be proved even there might be medicines that are made from *Cicer arietinum*.

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